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13. ABSTRACT (Maximum 200 Words)

The main objective of this project is to evaluate the effects of soy phytoestrogen consumption on reproductive hormones and prostate tissue markers of cell proliferation and androgen action in men at high risk of prostate cancer. The hypothesis is that alteration of endogenous hormones is a mechanism by which soy phytoestrogens prevent prostate cancer. A randomized parallel arm study will be performed, in which 60 men at high risk of prostate cancer will be randomized to receive one of three dietary supplements for six months: 1) soy powder containing phytoestrogens; 2) phytoestrogen-free soy powder; or 3) phytoestrogen-free milk powder. Urine and blood will be collected at 0, 3 and 6 months, for evaluation of prostate cancer risk factors, including serum hormones (testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone, estradiol, estrone, 3α, 17β-androstanediol glucuronide, sex hormone binding globulin) and prostate specific antigen, as well as urinary estrogen and phytoestrogen metabolites. Before and after the intervention, prostate biopsies will be performed to evaluate prostate tissue expression of apoptosis (TUNEL assay, Bax, Bcl-2), proliferation (Ki67, PCNA), and androgen receptor density. At this point, urine and blood from 21 subjects have been analyzed. 32 subjects have completed the study and 6 are enrolled, and biological samples have been collected, processed and stored. Immunohistochemistry will be performed summer 2005. We are requesting a one-year no cost extension in order to complete the subject enrollment, sample collection and all analyses.

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INTRODUCTION

The low risk of prostate cancer in Asia is thought to be due to dietary factors, including soy consumption. Studies showing an inverse association between prostate cancer risk and urinary excretion of soy phytoestrogens suggest that phytoestrogens contribute to the cancer-preventive effects of soy. One mechanism by which soy phytoestrogens are thought to be cancer-preventive is *via* reduction of endogenous sex hormones known to stimulate prostate cell growth. Despite the interest in soy phytoestrogens for prevention of prostate cancer, there have been no studies in men to evaluate the effects of soy phytoestrogen consumption on sex steroids and prostate tissue biomarkers, and no studies evaluating effects of phytoestrogen metabolism on sex steroids in men.

The main objective of this project is to evaluate the effects of soy phytoestrogen consumption on reproductive hormones and prostate tissue markers of cell proliferation and androgen action in men at high risk of prostate cancer. The underlying hypothesis is that alteration of endogenous hormones is a mechanism by which soy phytoestrogens prevent prostate cancer.

The specific aims of this study are to compare the effects of consumption of phytoestrogen-containing soy protein, phytoestrogen-free soy protein, and milk protein, on risk factors for prostate cancer (endogenous hormones, prostate specific antigen, prostate tissue markers of cell proliferation and hormone action), in men at high risk for prostate cancer. Comparing the three groups will enable us to distinguish the specific effects of soy phytoestrogens from effects caused by other soy components. A randomized parallel arm study will be performed, in which 63 men at high risk of prostate cancer will be randomized to receive one of three dietary supplements for six months: 1) soy powder containing 1 mg phytoestrogens/kg body weight; 2) phytoestrogen-free soy powder; and 3) phytoestrogen-free milk powder. Urine and blood will be collected at 0, 3 and 6 months, for evaluation of serum hormones (testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone, estradiol, estrone, 3α, 17β-androstanediol glucuronide, sex hormone binding globulin) and prostate specific antigen, as well as urinary estrogen and phytoestrogen metabolites. Before and after the intervention, prostate biopsies will be performed to evaluate prostate tissue expression of apoptosis (TUNEL assay, Bax, Bcl-2), proliferation (Ki67, PCNA), and androgen receptor density.

Data from *in vitro*, animal and epidemiological studies suggest that androgens and estrogens play a role in prostate carcinogenesis. Soy phytoestrogens have been shown to alter sex steroids in women in a potentially beneficial direction, yet such studies in men have not been reported. Studies of the hormonal effects of soy phytoestrogens in men will contribute to our knowledge of the cancer-preventive mechanisms of soy phytoestrogens, and may lead to dietary recommendations for prevention of prostate cancer.

BODY

According to the original statement of work, the following tasks were to be performed during the three years of this project:

Task 1: Hire and train staff, coordinate with Veteran's Administration and Fairview-University Hospital staff, establish all study protocols

Task 2: Perform feeding study on 90 men

- Recruit 90 men at high risk of prostate cancer and randomize into three intervention groups: phytoestrogen-containing soy protein (Soy +), phytoestrogen-free soy protein (Soy-), or milk protein
- Perform feeding study; process and store serum, urine and biopsy slides
- Analyze samples: serum hormones and SHBG by RIA; serum free and total PSA by ELISA; urine estrogen metabolites and phytoestrogens by GC-MS; biopsy slides by immunohistochemistry

Although the grant officially began on April 15, 2002, final approval from the DOD IRB was not received until January 2003. As a result, we were not able to begin recruiting subjects until February 2003 and began the study about one year late.

From February-April 2003, six subjects began the feeding study. From May 2003-April 2004, 30 subjects were enrolled in the study. From May 2004-April 2005 26 subjects were enrolled in the study. Of these 62 subjects, 7 withdrew (11.3%) for the following reasons:

- Dislike of powder taste (2 on Soy +, 1 on Soy -)
- Difficulty remembering to take powder
- Concern about weight gain
- Diagnosed prostate cancer patient elected medical treatment
- Relocated for his work

Of the 62 subjects enrolled, another 17 subjects never began the study after consenting (27%) for the following reasons:

- Dislike of powder taste (3 on milk, 2 on Soy +, 1 on Soy -)
- Gastrointestinal problems with powder (4 on milk , 2 on Soy +)
- Consumption of excess alcohol
- Type II diabetic on weight loss program
- Difficulty with time commitment (n = 4)
- Unwillingness to drive to study site from out of town home
- Thought there would be monetary compensation for participation
- Called back into active duty

As of May 2004, 10 subjects had completed the study and 17 were enrolled. As of May 2005, 32 subjects have completed the study and 6 are enrolled. Thus, 22 subjects completed the study during this past year. We are a little more than one year behind in progress. This is not surprising given the late start due to the time required for DOD IRB approval.

Clearly we did not anticipate the difficulty we would face in recruiting and retaining subjects. The main cause has been reduced PSA screening, which has resulted in far fewer biopsies, and a much slower recruiting rate than initially planned. We also did not anticipate the very high dropout rate.

In order to increase subject recruitment and retention, we have done the following:

- Increased the age range to 50-85 years
- Provided travel reimbursements for subjects who live out of town
- Included subjects who had negative biopsies within the past 2 years (as opposed to the past 6 months as originally planned)
- Increased flexibility in setting appointments

As a result of these changes, we doubled subject accrual from 2 subjects/month in 2003 to 4 subjects/month in 2004. In order to attempt to achieve our goal of 90 subjects, we further widened the inclusionary criteria by

- Including subjects with prostate cancer who are undergoing "watchful waiting"
- Allowing the subjects to miss their 3 month appointment
- Extending the study to 7 month for subjects who travel from Minnesota
- Shipping the soy protein powder to the winter home as necessary
- Remaining persistent in communication despite the distance
- Being as flexible as possible with appointments

Finally, we applied to the DOD for additional funding through the IDEA Award program ("Dietary Phytoestrogens and Prostate Cancer Prevention: Phase 2" PC041141), in order to include patients whose PSA is sufficiently high to recommend an initial biopsy, but who might not require a second biopsy. For these patients, we requested funds to pay for the second biopsy and provide compensation to the subjects as well. Funds were also requested to provide compensation to all subjects for participation and to add two community sites for recruitment (Park Nicollet Health Services and Metro Urology). Unfortunately, the grant was not awarded.

Despite this, we have acquired other funds to offer monetary compensation to the subjects and we are allowing spot urine collections from participants unwilling to collect 24 hours. We are hopeful these changes will increase subject recruitment.

In addition, power calculations have been performed under the guidance of Dr. William Thomas, our collaborator and biostatistician, in order to determine the minimum number of subjects that will be required to be able to detect significant effects. Using serum testosterone as the main endpoint, 21 subjects in each arm would allow us to detect a 16% to 33% change in serum total testosterone The difference detected would be 118 ng/dL which would be 16% in a man at the high end of normal and 33% in a man on the low end of normal. Thus we propose to lower our total number of subjects completing the study from 90 to 63. We believe that this is a more feasible and adequate number of subjects.

We are confident that we will be able to recruit 63 subjects and analyze all biological samples during the next year.

KEY RESEARCH ACCOMPLISHMENTS

- Enrollment of 62 subjects, completion of 31 subjects
- Collection, processing and storage of tissue, blood, and urine samples
- Analysis of blood and urine samples from 21 subjects.
- Improvement of the recruitment strategies

REPORTABLE OUTCOMES

PRELIMINARY DATA:

At this point, we have analyzed blood hormones and urinary estrogens and phytoestrogens from 21 subjects. The characteristics of these subjects are as follows:

	Change from Baseline		
	Soy + isoflavones n = 7	**Soy - isoflavones n = 7**	**Milk n = 7**
Total Testosterone	0.29 ± 1.0	0.50 ± 1.0	-0.46 ± 0.68
Estradiol	0.67 ± 4.1[ab]	2.4 ± 3.5[b]	-2.7 ± 3.6[a]
Estrone	-0.26 ± 24	7.3 ± 9.9	-7.2 ± 8.0
Free Testosterone	0.45 ± 1.7	-0.06 ± 0.97	-0.29 ± 1.6
Androstanediol Glucuronide	-0.66 ± 7.1	-0.54 ± 2.1	-1.08 ± 2.7
DHEAS	364 ± 1180	267 ± 307	-54 ± 40*
Androstenedione	-0.03 ± 0.24	-.001 ± 0.17	0.29 ± 0.25

Data are mean ± standard deviation. Pairwise comparisons are within rows: means that do not share letters are significantly different ($p < 0.05$).

The blood hormone data for the first 21 subjects are shown below:

	Change from Baseline		
	Soy + isoflavones n = 7	**Soy - isoflavones n = 7**	**Milk n = 7**
Total Testosterone	0.29 ± 1.0	0.50 ± 1.0	-0.46 ± 0.68
Estradiol	0.67 ± 4.1[ab]	2.4 ± 3.5[b]	-2.7 ± 3.6[a]
Estrone	-0.26 ± 24	7.3 ± 9.9	-7.2 ± 8.0
Free Testosterone	0.45 ± 1.7	-0.06 ± 0.97	-0.29 ± 1.6
Androstanediol Glucuronide	-0.66 ± 7.1	-0.54 ± 2.1	-1.08 ± 2.7
DHEAS	364 ± 1180	267 ± 307	-54 ± 40*
Androstenedione	-0.03 ± 0.24	-.001 ± 0.17	0.29 ± 0.25

Data are mean ± standard deviation. Pairwise comparisons are within rows: means that do not share letters are significantly different ($p < 0.05$).
*Significant within-group change from baseline ($p < 0.05$).

Double antibody radioimmunoassays (Diagnostics Systems Laboratory, INC., Webster, TX) using I^{125} were performed in duplicate with intra-assay variability of less than 5%. Data were analyzed using SAS Proc GLM (SAS version 8.2, SAS Institute, Inc., Cary, NC). An F-test comparing mean hormone differences among all hormones according to treatment status found significant mean estradiol differences ($F^* = 3.43$, $p = 0.05$) and a

trend in mean total testosterone differences (F* = 2.15, $p = 0.145$). All other mean hormone differences were not statistically significant ($p > 0.05$).

The treatment group *Soy - isoflavones* had the largest mean estradiol increase (mean = 2.40, sd = 3.5), followed by *Soy + isoflavones* (mean = 0.66, sd = 4.06). The *Milk* group had a decrease in estradiol concentration (mean = -2.74, sd 3.59). The *Soy + isoflavones* was significantly greater estradiol concentration change than the milk group ($p = 0.02$). Yet, *Soy - isoflavones* did not have mean estradiol differences significantly different than *Soy + isoflavones* ($p = 0.40$).

Dehyroepiandrostenedione-sulfate (DHEAS) decreased significantly from baseline in the milk treatment group only (t = 3.6, $p = 0.01$) All other hormone differences were not significantly different from baseline for any of the three treatment groups as determined by paired *t*-tests ($p > 0.05$).

These results are of course preliminary. The data analyses were performed mainly to set up and test the statistical program.

Perhaps most importantly, for the first time, we are able to report that urinary estrogens, thought to influence breast cancer risk, are found at significant levels in the urine of men at high risk of prostate cancer. In fact, for most of the estrogen metabolites, the levels fall within the range of pre- and postmenopausal women. Below are the data for estrogen metabolites and phytoestrogens for the first 21 subjects.

	% Subjects with Detectable levels	Range (ng/mL)
Estradiol	100%	48.7-5.4
Estrone	100%	8.1-1.5
4MeOE2	24%	15.9-0.5
4MeoE1	88%	8.7-0.5
2MeOE2	100%	15.2-1.5
2MeoE1	81%	6.2-0.5
16aOHE1	85%	7.8-0.5
Estriol	85%	26.9-0.5
2-OH-E2	38%	1.9-0.5
2-OH-E1	85%	4.9-0.5
4-OH-E1	23%	5.5-0.5
ODMA	98%	4-1090
Equol	96%	3-1790
Enterodiol	100%	2-780
Enterolactone	100%	13-1380
Daidzein	100%	10-5180
Dihydrodaidzein	16%	206-2660
Genistein	100%	1-1610
Glycitein	98%	1-1515
Matairesinol	96%	1-186

These results are highly significant. Given the suggested association of specific estrogen metabolites with breast cancer risk, a finding of similar levels in men suggests that studies should be performed in which these metabolites are measured in relation to prostate cancer risk.

CONCLUSIONS

The human feeding study is continuing and biological samples have been processed and are being stored as stated in the study design. At this point, urine and blood samples have been analyzed from 21 subjects for hormones and phytoestrogens. Immunohistochemistry will be performed on these subjects summer 2005.

Power calculations have caused us to lower our final number of subjects required from 90 to 63.

The most important finding to date is that estrogen metabolites that have been associated with breast cancer are found in men at levels similar to those in pre- and postmenopausal women. This suggests that these same metabolites may be of importance with respect to prostate cancer risk.

Given that the project began one year late, we request a one-year no-cost extension during which we are confident that we will complete the project.

REFERENCES

None

APPENDICES

None